INTRODUCTION

More than 500 disinfection by-products (DBPs) have been reported in the literature for the major disinfectants currently used (chlorine, ozone, chlorine dioxide, chloramines), as well as their combinations (Richardson, 1998). Of these reported DBPs, only a small percentage have been quantified in drinking waters. Thus, there is significant uncertainty over the identity and levels of DBPs that people are actually exposed to in their drinking water. Moreover, only a limited number of DBPs have been studied for adverse health effects. To determine whether the other DBPs pose an adverse health risk, more comprehensive quantitative occurrence and toxicity data are needed. To address this issue, scientists at the U.S. Environmental Protection Agency's (USEPA's) National Exposure Research Laboratory (NERL) initiated a proposal for a Nationwide DBP Occurrence Study.

Due to the large number of DBPs identified in drinking waters in the United States and other countries, it is not feasible to quantify all of them, so a way of prioritizing them was needed. Prior to this occurrence study, a multidisciplinary group of experts from the USEPA Office of Water and the USEPA Office of Prevention, Pesticides, and Toxic Substances had initiated a prioritization effort for the >500 DBPs reported in the literature according to their predicted adverse health effects (Woo et al., 2002). An in-depth, mechanism-based, structural activity relationship (SAR) analysis, supplemented by an extensive literature search for genotoxicity and other data, was used to rank the carcinogenic potential of these DBPs. Approximately 50 DBPs that received the highest ranking for potential toxicity, and that were not already included in the USEPA's Information Collection Rule (ICR), were selected for this occurrence study. Those ~50 DBPs are denoted 'high priority' DBPs in this report.

The 'high priority' DBPs include brominated, chlorinated, and iodinated species of halomethanes, brominated and chlorinated forms of haloacetonitriles, haloketones, haloacids, and halonitromethanes, as well as analogues of MX [3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone] (Table 1). Chemical Abstract Services (CAS) numbers are provided in Table 1 when they were available. Previously, MX had been determined to be the most mutagenic (to *Salmonella* bacteria) DBP ever identified in drinking water, accounting for as much as 20-50% of the total mutagenic activity measured in chlorinated drinking water samples (Kronberg and Vartiainen, 1988; Backlund et al., 1988; Meier et al., 1987). MX has also been shown to be carcinogenic in laboratory animals (Komulainen et al., 1997). Yet, very little drinking water occurrence data has been obtained for MX, so its potential hazard to humans has not been determined. There have also been recent reports of brominated DBP forms of MX (BMXs) (Suzuki and Nakanishi, 1995). These brominated DBP species are of concern because brominated species of DBPs have been shown to be significantly more carcinogenic than their chlorinated analogues. Brominated nitromethanes have also been recently shown to be extremely cytotoxic and genotoxic in mammalian cells (Plewa et al., 2002; Kargalioglu et al., in press). Specifically, they have been shown to be at least an order of magnitude more genotoxic to mammalian cells than MX and have genotoxicities greater than all of the regulated DBPs, except for monobromoacetic acid. It is interesting that dibromonitromethane and

bromonitromethane received the highest priority ranking of all DBPs in the SAR toxicity analysis effort.

It should be noted that Table 1 lists the identity of more than 50 high priority target species. During method development, additional species in the same analyte group were included for some of the drinking water plant surveys.

Because most of the high priority DBPs were from chlorine or chloramine disinfection, a few additional ozone and chlorine dioxide DBPs that were not ranked as a high priority were also included for completeness (i.e., to provide more information on those alternative disinfectants). In addition, methyl *tert*-butyl ether (MtBE) and methyl bromide, which are volatile organic compounds (VOCs) but not DBPs, were included in the list of target analytes because they are important source water pollutants, and their measurement would provide valuable occurrence information. Regulated and some ICR DBPs were also included in this study for comparison purposes (Table 2). In addition, routine water quality measurements, such as total organic carbon (TOC), total organic halide (TOX), assimilable organic carbon (AOC), and bromide were determined.

Table 1. Priority DBPs selected for Nationwide Occurrence Study [a]

MX and MX-Analogues:
3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX)
3-Chloro-4-(dichloromethyl)-2-(5H)-furanone (red-MX)
(E)-2-Chloro-3-(dichloromethyl)-butenedioic acid (ox-MX)
(E)-2-Chloro-3-(dichloromethyl)-4-oxobutenoic acid (EMX)
2,3-Dichloro-4-oxobutenoic acid (Mucochloric acid) [87-56-9]
3-Chloro-4-(bromochloromethyl)-5-hydroxy-2(5H)-furanone (BMX-1) [132059-51-9]
3-Chloro-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone (BMX-2) [132059-52-0]
3-Bromo-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone (BMX-3) [132059-53-1]
(E)-2-Chloro-3-(bromochloromethyl)-4-oxobutenoic acid (BEMX-1) [c]
(E)-2-Chloro-3-(dibromomethyl)-4-oxobutenoic acid (BEMX-2) [c]
(E)-2-Bromo-3-(dibromomethyl)-4-oxobutenoic acid (BEMX-3) [c]

Haloacids:
3,3-Dichloropropenoic acid

Halomethanes:
Chloromethane [74-87-3]
Bromomethane (methyl bromide) [74-83-9] [b]
Dibromomethane [74-95-3]
Bromochloromethane [74-97-5]
Bromochloroiodomethane [34970-00-8]
Dichloroiodomethane [594-04-7]
Dibromoiodomethane [c] [593-94-2]
Chlorodiiodomethane [c] [638-73-3]
Bromodiiodomethane [c] [557-95-9]
Iodoform [75-47-8] [c]
Chlorotribromomethane [594-15-0]
Carbon tetrachloride [56-23-5]

Halonitromethanes:
Bromonitromethane [563-70-2]
Chloronitromethane [c] [1794-84-9]
Dibromonitromethane [598-91-4]
Dichloronitromethane [c] [7119-89-3]
Bromochloronitromethane [c] [135531-25-8]
Bromodichloronitromethane [c] [918-01-4]
Dibromochloronitromethane [c] [1184-89-0]
Tribromonitromethane (bromopicrin) [c] [464-10-8]

Table 1 (Continued)

Haloacetonitriles:
Bromoacetonitrile [590-17-0]
Chloroacetonitrile [107-14-2]
Tribromoacetonitrile [75519-19-6]
Bromodichloroacetonitrile [60523-73-1]
Dibromochloroacetonitrile [144772-39-4]

Haloketones:
Chloropropanone [78-95-5]
1,3-Dichloropropanone [534-07-6]
1,1-Dibromopropanone
1,1,3-Trichloropropanone [921-03-9]
1-Bromo-1,1-dichloropropanone
1,1,1,3-Tetrachloropropanone [16995-35-0]
1,1,3,3-Tetrachloropropanone [632-21-3]
1,1,3,3-Tetrabromopropanone [c] [22612-89-1]
1,1,1,3,3-Pentachloropropanone [1768-31-6]
Hexachloropropanone [116-16-5]

Haloaldehydes:
Chloroacetaldehyde [107-20-0]
Dichloroacetaldehyde [70-02-7]
Bromochloroacetaldehyde [c] [98136-99-3]
Tribromoacetaldehyde [115-17-3] [c]

Haloacetates:
Bromochloromethyl acetate [247943-54-0]

Haloamides:
Monochloroacetamide [79-07-2] [c]
Monobromoacetamide [683-57-8] [c]
Dichloroacetamide [683-72-7]
Dibromoacetamide [c] [598-70-9]
Trichloroacetamide [594-65-0] [c]

Table 1 (Continued)

Non-Halogenated Aldehydes and Ketones:
2-Hexenal [505-57-7]; [6728-26-3]
5-Keto-1-hexanal [d]
Cyanoformaldehyde [4471-47-0]
Methylethyl ketone (2-butanone) [78-93-3] [d]
6-Hydroxy-2-hexanone [d]
Dimethylglyoxal (2,3-butanedione) [431-03-8]
Volatile organic compounds (VOCs) and Miscellaneous DBPs:
1,1,1,2-Tetrabromo-2-chloroethane
1,1,2,2-Tetrabromo-2-chloroethane [c]
Methyl-*tert*-butyl ether [1634-04-4] [b]
Benzyl chloride [100-44-7]

[a] Chemical Abstracts Services (CAS) numbers provided in brackets when available.
[b] Not a DBP, but included because it is an important source water contaminant.
[c] DBP not originally prioritized (identified in drinking water after initial prioritization), but included due to similarity to other priority compounds.
[d] DBP not given a high priority, but included for completeness sake to provide more representation to ozone DBPs for occurrence.

Table 2. Information Collection Rule and regulated DBPs included for comparison [a]

Halomethanes	Haloacetic acids (cont).
Chloroform	Dibromoacetic acid
Bromodichloromethane	Trichloroacetic acid
Dibromochloromethane	Bromodichloroacetic acid
Bromoform	Dibromochloroacetic acid
	Tribromoacetic acid
Haloacetonitriles	
Dichloroacetonitrile	Halonitromethanes
Bromochloroacetonitrile	Chloropicrin (trichloronitromethane)
Dibromoacetonitrile	
Trichloroacetonitrile	Haloaldehydes
	Chloral hydrate
Haloketones	(trichloroacetaldehyde)
1,1-Dichloropropanone	
1,1,1-Trichloropropanone	Oxyhalides
	Bromate
Haloacetic acids	Chlorate
Monochloroacetic acid	Chlorite
Monobromoacetic acid	
Dichloroacetic acid	
Bromochloroacetic acid	

[a] Five HAAs are regulated; six HAAs were required in the ICR, however some utilities reported data on the complete set of 9 HAAs.

The design of this study involved the study of drinking waters disinfected with the four common disinfectants: chlorine, chloramines, ozone, and chlorine dioxide. Because many of the high priority DBPs were brominated, it was important to include drinking waters that contained relatively high bromide levels. In addition, many of the waters selected for study were relatively high in TOC. Drinking water samples were selected from across the United States to assess the distribution and speciation of by-products in a variety of different waters from geographically diverse regions, with differing water quality, treatment, and distribution system characteristics (Figure 1). Moreover, pairs of treatment plants were chosen that used source waters from the same (or similar) watersheds but employed different treatment technologies and disinfection scenarios. This permitted an evaluation of the impact of technology and disinfectant combinations on by-product formation, while minimizing confounding factors related to differing source water quality. Each of the plants provided operational information and complementary water quality analyses. Drinking water was also sampled at typically two points in each distribution system to determine the fate and transport of DBPs—as well as actual occurrence in the distribution system—and simulated distribution system (SDS) tests were conducted to determine the formation and stability of DBPs in the presence of chlorine or chloramines. Previously, most of the newly identified DBPs were detected in drinking waters that had been sampled only at the treatment plant; very little was known about the fate and transport (and stability) of most of the newly identified DBPs in the distribution system. To this end, the influence of water quality parameters, treatment, and distribution system conditions on DBP concentrations and persistence (stability) was a major objective of this work. The drinking water utilities that were sampled are shown in Table 3.

Sampling Survey: 12 plants sampled quarterly

2 plants - same watershed - different treatment/disinfection

Plants sampled in EPA Regions 3, 4, 5, 6, 7, and 9

Figure 1. Sampling survey.

Table 3. Drinking water utilities sampled

Utility[a] (EPA Region[b])	Disinfection Used
Plant 1 (EPA Region 9)	Ozone-chlorine-chloramines
Plant 2 (EPA Region 9)	Chlorine-chloramines
Plant 12 (EPA Region 6)	(Chlorine dioxide-)Chloramines
Plant 11 (EPA Region 6)	Chlorine dioxide-chlorine-chloramines
Plant 8 (EPA Region 4)	Chlorine-chloramines
Plant 7 (EPA Region 4)	Chloramines-ozone
Plant 6 (EPA Region 4)	Chlorine dioxide-chlorine-chloramines
Plant 5 (EPA Region 4)	Ozone-chlorine
Plant 3 (EPA Region 3)	Chlorine-chloramines
Plant 4 (EPA Region 3)	Chlorine
Plant 10 (EPA Region 5)	Chlorine-chloramines
Plant 9 (EPA Region 7)	Chlorine-chloramines

[a]The following pairs of plants treated water from the same or similar watersheds: plants 1and 2; 3 and 4; 5 and 6; 7 and 8; 9 and 10; and 11 and 12**.**

[b]The 12 plants in this survey were located in six of the nine regions defined by the EPA. The states included in each of these six regions are as follows:

EPA Region 9—Arizona, California Hawaii, Nevada
EPA Region 6—Arkansas, Louisiana, New Mexico, Oklahoma, Texas
EPA Region 4—Alabama, Florida, Georgia, Kentucky, Mississippi, North Carolina, South Carolina, Tennessee
EPA Region 3—Delaware, Maryland, Pennsylvania, Virginia, West Virginia, Washington D.C.
EPA Region 5—Illinois, Indiana, Michigan, Minnesota, Ohio, Wisconsin
EPA Region 7—Iowa, Kansas, Missouri, Nebraska

Because there were no existing quantitative analytical methods for most of the high priority DBPs, methods were initially developed at UNC and MWDSC. The high priority DBPs were divided between UNC and MWDSC for method development and quantitative analyses (UNC measured the MX analogues, carbonyls, 3,3-dichloropropenoic acid, haloacetates, haloamides, and some haloaldehydes; MWDSC measured bromate, chlorate, chlorite, halomethanes, haloacetic acids, haloacetonitriles, haloacetaldehydes, haloketones, halonitromethanes, methyl ethyl ketone, methyl *tertiary* butyl ether (MTBE), tetrabromochloroethane, and benzyl chloride). In addition, a method was used at UNC for differentiating the total organic chlorine and bromine. No one single analytical method could be used for all DBPs, so different methods were developed and optimized for specific groups of DBPs. Also, because there were no commercially available standards for many of these compounds, a significant number had to be synthesized. A combination of extraction and derivatization techniques were utilized that minimized artifact formation and maximized recovery of the target analytes from the aquatic matrix. Positive identification was achieved through use of a combination of complementary spectroscopic tools, some of which were designed to target a broader range of by-products than those listed, and/or dual-column gas chromatography. Once methods for the target by-products were established, studies of their formation and stability were conducted at full-scale treatment plants and their respective distribution systems.

Another goal of this project was to use this opportunity to look for other DBPs that had not been previously identified in order to provide a more complete assessment of DBPs formed by different treatments in different regions of the U.S. This work was carried out at the USEPA NERL-Athens laboratory. For this research, a combination of mass spectrometric techniques (gas chromatography with high and low resolution electron ionization mass spectrometry, and with chemical ionization mass spectrometry) was used to aid in the identification of these new DBPs. Mass spectra for those DBPs that had not been previously reported (i.e., those identified in this study for the first time) are provided in the Appendix of this report.

Presentations of preliminary results from this Nationwide DBP Occurrence Study have been given at several scientific meetings over the last three years. Citations of the more comprehensive proceedings articles appear below for reference (Krasner et al., 2002; Sclimenti

et al., 2002; Krasner et al., 2001; Weinberg et al., 2001; Gonzalez et al., 2000; Onstad et al., 2000, Onstad and Weinberg, 2001).

This report is presented in multiple chapters, each of which represents a specific component of the research, method development, and DBP analysis in the treatment plants after different unit processes and/or disinfectant addition and in the distribution systems.

REFERENCES

Backlund, P., L. Kronberg, and L. Tikkanen. Formation of Ames mutagenicity and of the strong bacterial mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone and other halogenated compounds during disinfection of drinking water. *Chemosphere* 17(7): 1329 (1988).

Gonzalez, A. C., S. W. Krasner, H. Weinberg, and S. D. Richardson. Determination of newly identified disinfection by-products in drinking water. *Proceedings of the American Water Works Association Water Quality Technology Conference*, American Water Works Association: Denver, CO, 2000.

Kargalioglu, Y., E. D. Wagner, S. D. Richardson, and M. J. Plewa. DNA damage in the CHO/Comet assay induced by nitrohalomethanes, a novel class of drinking water disinfection by-products. *Environmental Science & Technology* (in press).

Komulainen, H., V.-M. Kosma, S.-L. Vaittinen, T. Vartiainen, E. Kaliste-Korhonen, S. Lotjonen, R. K. Tuominen, and J. Tuomisto. Carcinogenicity of the drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone in the rat. *Journal of the National Cancer Institute* 89(12): 848 (1997).

Krasner, S. W., R. Chinn, S. Pastor, M. J. Sclimenti, S. D. Richardson, A. D. Thruston, Jr., and H. S. Weinberg. Relationships between the different classes of DBPs: formation, speciation, and control. *Proceedings of the American Water Works Association Water Quality Technology Conference*, American Water Works Association: Denver, CO, 2002.

Krasner, S. W., S. Pastor, R. Chinn, M. J. Sclimenti, H. S. Weinberg, and S. D. Richardson. The occurrence of a new generation of DBPs (beyond the ICR). *Proceedings of the American Water Works Association Water Quality Technology Conference*, American Water Works Association: Denver, CO, 2001.

Kronberg, L., and T. Vartiainen. Ames mutagenicity and concentration of the strong mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone and of its geometric isomer E-2-chloro-3-(dichloromethyl)-4-oxo-butenoic acid in chorine-treated tap waters. *Mutation Research* 206:177 (1988).

Meier, J. R., R. B. Knohl, W. E. Coleman, H. P. Ringhand, J. W. Munch, W. H. Kaylor, R. P. Streicher, and F. C. Kopfler. Studies on the potent bacterial mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone: aqueous stability, XAD recovery and analytical determination in drinking water and in chlorinated humic acid solutions. *Mutation Research* 189:363 (1987).

Onstad, G. D., H. S. Weinberg, S. W. Krasner, and S. D. Richardson. Evolution of analytical methods for halogenated furanones in drinking water. *Proceedings of the American Water*

Works Association Water Quality Technology Conference, American Water Works Association: Denver, CO, 2000.

Onstad, G. D., and H. S. Weinberg. Improvements in extraction of MX-analogues from drinking water. *Proceedings of the American Water Works Association Water Quality Technology Conference*, American Water Works Association: Denver, CO, 2001.

Plewa, M. J., E. D. Wagner, and S. D. Richardson. Quantitative comparative mammalian cell cytotoxicity and genomic genotoxicity of drinking water disinfection by-products. Paper presented at the *International Society of Exposure Analysis (ISEA)-International Society for Environmental Epidemiology (ISEE) Conference*, Vancouver, Canada, August 11-15, 2002.

Richardson, S. D. Drinking water disinfection by-products. In *The Encyclopedia of Environmental Analysis and Remediation* (R.A. Meyers, ed.), Vol. 3, John Wiley & Sons: New York, 1998, pp.1398-1421.

Sclimenti, M. J., S. W. Krasner, and S. D. Richardson. The determination of DBPs using a solid phase microextraction (SPME)-GC/ECD technique. *Proceedings of the American Water Works Association Water Quality Technology Conference*, American Water Works Association: Denver, CO, 2002.

Suzuki, N., and J. Nakanishi. Brominated analogues of MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone in chlorinated drinking water. *Chemosphere* 30(8):1557 (1995).

Weinberg, H. S., S. W. Krasner, and S. D. Richardson. Determination of new carbonyl-containing disinfection by-products in drinking water. *Proceedings of the American Water Works Association Water Quality Technology Conference*, American Water Works Association: Denver, CO, 2001.

Woo, Y.-T., D. Lai, J. L. McLain, M. K. Manibusan, and V. Dellarco. Use of mechanism-based structure-activity relationships analysis in carcinogenic potential ranking for drinking water disinfection by-products. *Environmental Health Perspectives* 110(Suppl. 1):75 (2002).